IN THE SPECIFICATION:

Please replace the paragraph on page 3, lines 14-34, with the following rewritten paragraph:

T-regulatory cells have an important role in peripheral tolerance, but it has been difficult to isolate cells with suppressive activity in vitro and to define their mechanism of action. A $CD4^+$ T-regulatory cell subset has been described which is able to suppress antigen-specific immune responses in vitro and in vivo. See, e.g., ~~USSN 07/846,208, filed March 4, 1992; USSN 08/643,810, filed May 6, 1996~~ U.S. Pat. No. 6,277,635; and Groux, et al. (1997) Nature 389:737-742; each of which is incorporated herein by reference. Type 1 T-regulatory (Tr1) cells are defined, in part, by their unique cytokine profile: they produce high levels of IL-10, significant levels of TGF-β and IFN-γ, but no IL-4 or IL-2. Herein, it is investigated whether in vitro differentiation of human Tr1 cells from naive $CD4^+$ T cells is regulated by cytokines. It is shown that in cord blood T cells, IFN-α induces differentiation of a population of cells with a Tr1-like profile of cytokine production. In contrast, with peripheral blood T cells, both exogenous IL-10 and IFN-α were required for differentiation of Tr1 cells. Cultures with Tr1 cells had a reduced proliferative capacity in response to polyclonal activation, and a suppressed response to alloantigens. Suppression of the alloantigen response was mediated in part by IL-10 and TGF-β. The present invention is based, in part, on the definition of conditions for in vitro differentiation of human Tr1 cells. This will facilitate further characterization of this unique T-cell subset and enable their clinical use as cellular therapy to induce tolerance to foreign proteins, e.g., alloantigens.

Please replace the paragraph on page 14, lines 5-23, with the following rewritten paragraph:

A number of experiments were designed to determine the effects of IL-10, IFN-α, and IL-15 on the differentiation of IL-10-producing T cells. Efforts have focussed on aspects of the differentiation system described by Sornasse, et al. ~~(1996) J. Exp. Med. 184:473-xxx)~~ , supra, which involves co-culture of CD4+ T cells with irradiated L-cells,

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expressing CD32, CD58, and CD80, in the presence of anti-CD3, IL-2, and/or IL-15, and polarizing cytokines. Following two rounds of stimulation, cells are collected, stimulated with αCD3 and αCD28, and analyzed by intra-cytoplasmic staining and ELISA for the production of IL-10, IL-4, IL-2, and IFN-γ. Experiments were initiated with CD4+ T cells derived from cord blood, which cells have an innate ability to produce high levels of IL-10. Addition of IFN-α resulted in a significant, e.g., 5-6 fold, increase in the percentage of IL-10-positive cells compared to addition of IL-10 alone. Consistent with a Tr1 phenotype, approximately 50% of the IL-10 positive cells were also positive for IFN-γ. Further efforts are underway to determine the percentage of IL-10+IL-4- and IL-10+IL-2- cells at the single cell level. In order to definitively establish the percentage of Tr1 cells differentiated in vitro, we plan to perform limiting dilutions (after the secondary stimulation) in the presence of allogeneic feeder-cell mixture and to analyze the profile of cytokine production at the clonal level.

Please replace the paragraph on page 18, lines 18-25, with the following rewritten paragraph:

While certain responses of tolerance characterized as anergy result from blockage of signaling at the T cell receptor (see Weiss and Littman (1994) Cell 76:263-274; Chan, et al. (1994) Ann. Rev. Immunol. 12:555-592; and Fraser, et al. (1993) Immunol. Today 14:357-362), the anergy described herein exists with functional T cell receptor. In particular, stimulation with anti-CD3 still results in a Ca^{++} flux. But the antigen-specific T cells do not respond to the antigen stimulation in the normal manner, e.g., by production of cytokines and/or cell proliferation.

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Please replace the paragraph beginning on page 19, line 34, and continuing to page 20, line 17, with the following rewritten paragraph:

h-IL-10 inhibits the synthesis of IFN-γ and granulocyte-macrophage colony stimulating factor (GM-CSF) induced in human PBMC by PHA, anti-CD3 mAb, and IL-2 (Bacchetta, et al. ~~(1989) J. Immunol. 144:902~~ (1990) J. Immunol. 144:902-908; and Bevan (1984) Immunol Today 5:128. This inhibition occurs at the transcriptional levels (Altmann, et al. (1989) Nature 338:512-514; Bacchetta, et al., supra). Murine IL-10 (m-IL-10) has pleiotropic activities on different cell types, including growth promoting effects on thymocytes (Chen, et al. (1991) J. Immunol. 147:528-534), cytotoxic T cells (De Koster, et al. (1989) J. Exp. Med. 169:1191-1196), and mast cells (de Waal Malefyt, et al. (1991) J. Exp. Med. 174:1209-1220). m-IL-10 induces class II MHC antigen expression on B cells and sustains the viability of these cells (de Waal Malefyt, et al. (1991) J. Exp. Med. 174:915-924). Furthermore, IL-10 inhibits cytokine production by macrophages (Bejarano, et al. (1985) Int. J. Cancer 35:327-333; Fiorentino, et al. (1989) J. Exp. Med. 170:2081-2095). h- and m-IL-10 have extensive homology to BCRF-1, an open reading frame of the Epstein Barr virus (EBV) genome (Azuma, et al. (1992) J. Exp. Med. 175:353-360; Bacchetta, et al. ~~(1989) J. Immunol. 144:902~~ , supra). The protein product of BCRF-1, designated viral IL-10 (v-IL-10), shares most properties with h-and m-IL-10 including CSIF activity on human and mouse T cells (Bacchetta, et al., supra; Bevan, ~~M. J.,~~ supra).

Please replace the paragraph on page 20, lines 18-28, and replace with the following rewritten paragraph:

h-IL-10 and v-IL-10 inhibit antigen specific proliferative responses by reducing the antigen presenting capacity of human monocytes via downregulation of class II MHC molecules (Figdor, et al. (1984) J. Immunol. Methods 68:~~68~~ 73-87). Moreover, IL-10 inhibits cytokine synthesis by LPS or IFN-γ activated monocytes, including CM-CSF, G-CSF, and the proinflammatory cytokines IL-1α, IL-1β, IL-6, IL-8, and TNF-α

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(Bejarano, et al. (1985) Int. J. Cancer 35:327-333; Fiorentino, et al, supra.). Interestingly, LPS activated monocytes produce high levels of IL-10, and enhanced production of cytokines was observed in the presence of anti-IL-10 mAb indicating an autoregulatory effect of IL-10 on monokine production (Bejarano, et al., supra).

Please replace the paragraph beginning on page 20, line 31, and continuing to page 21, line 4, and replace with the following rewritten paragraph:

Alloreactivity reflects, at least in part, recognition of foreign MHC molecules plus antigenic peptides of various origin (Fiorentino, et al. (1991) J. Immunol. 146:3444-3451; Fiorentino, et al. (1991) J. Immunol. 147:3815-3822; Freedman, et al. (1987) J. Immunol. 139:3260-3267; Go, et al. (1990) J. Exp. Med. 172:1625-1631). Moreover, alloreactive T cells may recognize conformational differences between MHC molecules largely independent of the peptides bound, or even on empty MHC molecules (Harding, et al. (1990) Proc. Natl. Acad. Sci. USA 87:5553-5557; Hsu, et al. (1990) Science 250:830-832; Julius, et al. (1973) Eur. J. Immunol. 3:645-649). IL-10 inhibits allospecific proliferative responses, and cytokine production. In addition, the reduced proliferative responses could not be restored by exogenous IL-2.

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